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Chemical, biochemical and environmental aspects of atrazine

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Key words: Atrazine; toxicity; accumulation; decomposition; analytical methods.

Abstract

Atrazine herbicide is one of the primarily herbicide used for agricultural purposes worldwide. Amongst the different herbicide used more attention is paid to atrazine as it exhibit serious environmental problems including ecological risks and some human health damages. Due to their extensive usage and long half life period most of the actual evidence suggests that atrazine herbicide is omnipresent compounds found in different ecological compartments. Their highly hydrophilic character and low volatility have resulted in significant persistence in the aquatic environment. Very few studies describe the detailed study on fate and toxicity of atrazine herbicide in the environment. Here we review several important issues with regard to: (1) Laboratory based synthesis and mode of action on plant (2) the toxicity of atrazine herbicide (3) methods for determination in different biological systems and; (5) the by-products generated during chemical and biological decomposition. Based on recent research results the occurrence of atrazine herbicide in the environment inhibits the growth of some terrestrial aquatic species and leads to environmental risks. Most of the wastewater treatment plants are not capable of removing effectively the atrazine herbicide. Therefore there is a need to develop alternative processes to remove them from waters. Advanced oxidation processes have been proposed as alternative methods to ensure higher degradation and mineralization of atrazine herbicide are present in waters. Based on recent research results in the different biological media mass chromatography is considered as best tool for the determination of atrazine.

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Introduction

Over the past few decades the use of pesticides in the agriculture field for preserving crops for humans and animals has resulted in their undesirable accumulation in the environment (De Lorenzo *et al.*, 2001). Most of these herbicides are stable and difficult to oxidize by conventional treatment processes (Kross *et al.*, 1992; Hayes *et al.*, 2002; Krämer 2007). A significant number of these compounds have been frequently detected in different type of waters (wastewater surface water drinking water ground water) and solids (sludge soil and sediments) (Hayes *et al.*, 2002; Krämer 2007). Due to their excessive occurrences and their persistent characters in the environment there is a considerable interest for herbicides residues. The long-term and the low-dosed exposures to agriculture products in the environment lead to the adverse impacts to the target organisms including neurotoxic disruption endocrine disruption and chronic toxicity (Rusiecki *et al.*, 2004).

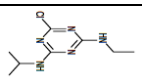
According to previous research (Kross *et al.*, 1992; Krämer 2007) heterocyclic herbicides can enter in the aquatic environment through the direct discharge of agriculture water stream and the discharge of effluents from wastewater treatment plants. The introduction of these residual compounds into environment through different sources will lead to serious environmental problems including ecological risk and human health damage.

History and physicochemical properties of atrazine

Atrazine (2-chloro-4-(ethylamino)-6-(isopropyla-mino)-s-triazine) is a selective systemic and most popular herbicide introduced in 1958 by J.R. Geigy (Hayes *et al.*, 2002; Krämer 2007). It has a range of trade names including Marksman, Coyote, Atrazina, Atrazol and Vectal. Atrazine is used for the pre and post-emergence control of annual and broad leaved weeds and perennial grasses; it inhibits photosynthesis and interferes with other enzymic processes (Hayes *et al.*, 2002; Chan *et al.*, 2005). It is mainly absorbed through the plant roots but can enter through the foliage and accumulates in the apical meristems and leaves. Globally atrazine is used in the production of maize sorghum sugar cane pineapples chemical fallows grassland macadamia nuts conifers and for industrial weed control with its biggest market in maize production (Ribeiro *et al.*, 2005).

The Octanol/Water Partition (Kow) Log Kow is 2.75 Solubility 0.03 g/L of water at 20°C. Soil sorption coefficient Koc average is 122; medium to high mobility in soil (Hayes *et al.*, 2002; Krämer 2007) (Table 1). Annually in the U.S.A. atrazine use has been estimated to be 40'000 tons and 5'000 tones in China. Before the ban of atrazine in France in 2003 10'000 tons of atrazine were spread on cultivated areas in this country and in India is approximately 1'000 tons per annum (EPA 2003).

Table 1. Structure and physicochemical properties of Atrazine.

Chemical structure	Molecular weight (g/mol)	Solubility (mol/L)	Log Kow	Henry's law constant (atm m³/mol)
	215.68	7 mg/100 mL in water at 25°C	2.2041 at 25°C	1.093291e-007 at 25°C

Toxicity of atrazine

Atrazine is slightly toxic to birds – there is no mortality at 10000 mg/kg diet virtually non-toxic for bees with an LD_{50} of >1000µg/bee and classified as moderately toxic for aquatic organisms (96-h LC_{50} range from 0.5-15mg/L) the LD_{50} for catfish is 7.6 mg/l and 4.3 mg/l for guppies (Kross *et al.*, 1992) (Table 2). The long exposures and improper handling of atrazine may leads to adverse effects there are some evidences regarding the exposure to atrazine associated with cancers in humans like lung bladder non-Hodgkin's lymphoma leukemia multiple myeloma ovarian cancer colon cancer (Rusiecki *et al.*, 2004) and reduced sperm quality in humans (Swan *et al.*, 2003).

Table 2. Toxicity of atrazine among the mammals, aquatic and plant species.

Mammals	Aquatic organisms	Plants
Rat- LD_{50} 3080 mg/kg, oral, >5600 mg/kg, dermal and LC_{50} > 710 mg/m3 (1h), inhalation	**Green algae**- EC_{50} 0.055 mg/l (inhibition of growth) and EC_0 0.030 mg/l (inhibition of cell division)	**Corn**- >70% effect at 4.5 kg/ha. **Wheat**- 35-70% effect at 4.3 mg/l
Hamster- LD_{50} 1000 mg/kg, oral	**Rainbow trout**- LC_{50} 8.8 mg/l (96h)	**Lettuce**- >70% effect at 0.5 kg/ha
Mouse- LD_{50} 1750 mg/kg, oral	Perch- LC_{50} 16 mg/l (96h)	**Millet**- 35-70% effect at 1.6 mg/l
Rabbit- LD_{50} 750 mg/kg, oral and 7500 mg/kg, dermal	**Carp**- LC_{50} 76 mg/l (96h)	**Soybean**- 35-70% effect at 1.1 mg/l

The disruptive effects of atrazine on endocrine activity has been suggested to occur via multiple mechanisms including inhibition of androgen receptors in mammals (Danzo 1997) disruption of the hypothalamic control of pituitary–ovarian function in mammals (Cooper *et al.*, 2000) alteration of corticosterone and thyroid hormones in amphibians (Larson *et al.* 1998) and by induction of aromatase that results in an increased conversion of androgen to estrogen in human cell lines (Sanderson *et al.*, 2000 and 2001) amphibians (Hayes *et al.*, 2003; Hayes *et al.*, 2002) and potentially in reptiles (Crain *et al.*, 1997).

Synthesis of Atrazine

Synthetic atrazine is prepared from cyanuric chloride (Fig. 1) which is treated sequentially with ethylamine and isopropyl amine and it is used in millions tones per years worldwide (Pearlman *et al.*, 1948; Chen *et al.*, 2013).

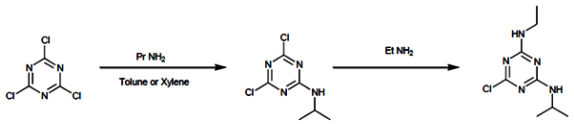


Fig. 1. Synthesis scheme of atrazine.

Mode of Action on Plants/Weeds

Photosystem II (PSII) in plants and bacteria (most importantly cyanobacteria) withdraws electrons from water and reduces plastoquione (PQ) using light energy. Upon light absorption charge separation takes place between the dimeric chlorophyll P680 and the pheophytin electron acceptor (Takano *et al.*, 2008). On the electron acceptor side the electron is transferred from pheophytin to the primary quinone electron acceptor QA and then to the secondary quinone acceptor QB. Herbicide targeting PSII is known to bind to the QB site and blocks the electron transfer beyond QA (Oettmeier 1999; Trebst 2007). From the studies of herbicide-resistant mutants it has been proposed that D1-Ser264 plays an important role in binding of atrazine in the QB pocket (Oettmeier 1999; Takano *et al.*, 2008). Atrazine functions by binding to the plastoquinone-binding protein in photosystem II as a results step process from plastoquinone to cytochrome (marked by cross sign) and reaming steps inhibited as highlighted in following flow steps (Fig. 2). Plant death results from starvation and oxidative damage caused by breakdown in the electron transport process. Oxidative damage is accelerated at high light intensity (Appleby *et al.*, 2001; EPA 2003; Takano *et al.*, 2008).

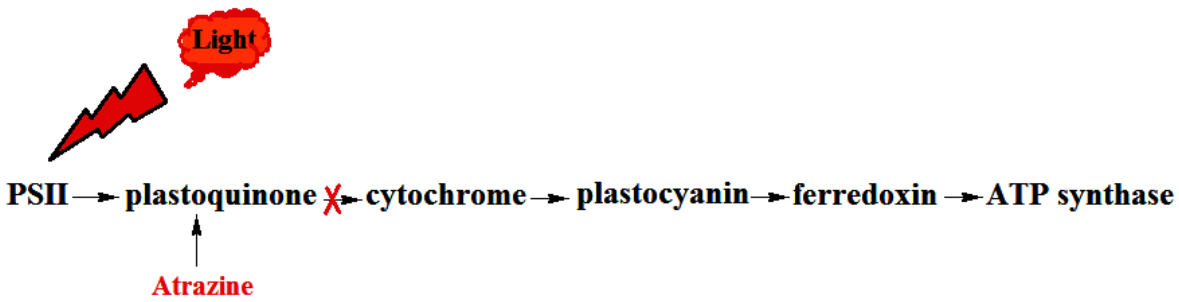


Fig. 2. Mode of action of atrazine.

Decomposition of Atrazine

It has been said that atrazine is degradable and has little tendency to bioaccumulate thereby limiting possible long-term adverse effects on fish and wildlife (EPA 2003; Hayes *et al.*, 2002; Krämer 2007). Atrazine is an invasive environmental contaminant strongly persistent and is one of the most significant water pollutants in rain surface marine and ground water. Its persistence and mobility in some types of soils because it is not easily absorbed by soil particles means it often causes contamination of surface and ground waters (Hayes *et al.*, 2002; Krämer 2007). Atrazine and its metabolites persistent presence had been a serious problem especially in surface and ground water systems (Gianessi *et al.*, 1997; EPA 2003). Atrazine is classified as a moderately persistent chemical with half-life ranging from several weeks to months in soil with an average of 40 days (EPA 2003). But atrazine is extremely persistent in clay and sandy loam soils at 15°C with half-life of 105 and 166 weeks respectively whereas half-life of atrazine and its derivatives has been shown to exceed 170 days in aquifer sediments (Gianessi *et al.*, 1997; Radosevich *et al.*, 1989).

Atrazine is moderately soluble in water. Chemical hydrolysis followed by biodegradation may be the most important route of disappearance from aquatic environments. Hydrolysis is rapid under acidic or basic conditions but is slower at neutral pHs. Atrazine is not expected to strongly adsorb to sediments. Bioconcentration and volatilization of atrazine are not environmentally important (Howard *et al.*, 1989). Atrazine is absorbed by plants mainly through the roots but also through the foliage. Once absorbed it is translocated upward and accumulates in the growing tips and the new leaves of the plant. In susceptible plant species atrazine inhibits photosynthesis. In tolerant plants it is metabolized (Kidd *et al.*, 1991). Most crops can be planted 1 year after application of atrazine. Atrazine increases the uptake of arsenic by treated plants (Wauchope *et al.*, 1992).

Chemical Decomposition of Atrazine

Atrazine may be chemically hydrolyzed forming the inactive hydroxyatrazine which in turn may be microbiologically decomposed. The half-life for hydrolysis is strongly dependent on the pH of the soil. In a neutral slightly alkaline or slightly acid environment atrazine is stable (half-life at pH 7-9: 10000 days). Hydrolysis mainly takes place under alkaline or acid conditions (half-life at pH 3 or pH 11 » 3 months) (Acosta *et al.*, 2004; Varghese *et al.*, 2007). Aqueous solutions of atrazine decompose upon illumination with a low-pressure Hg-arc lamp at 254 nm. However no decomposition takes place with more than 300 nm wavelength. On the other hand addition of polyoxometalates into a solution of atrazine photodecomposes the substrate within a few minutes at 320 nm.

Ultrasound treatment also decomposes aqueous solutions of atrazine within a few minutes (Dao *et al.*, 2011; De Veer *et al.*, 1994; Hiskia *et al.*, 2001; Varghese *et al.*, 2007). Both methods sonolysis and photolysis with polyoxometalates give common intermediates namely 2-hydroxy-4-(isopropylamino)-6-amino-s-triazine 2-chloro-4-(isopropylamino)-6-amino-s-triazine 2-chloro-4-amino-6-(ethylamino)-s-triazine 2-hydroxy-46-diamino-s-triazine and 2-hydroxy-4-hydroxy-6-amino-s-triazine among others. The final products for both methods US and photolysis with polyoxometalates were cyanuric acid NO^{3-} Cl^{-} CO_2 and H_2O. 2-hydroxy-4-hydroxy-6-amino-s-triazine showed no signs of decomposition by sonication and/or photolysis with polyoxometalates (Dao *et al.*, 2011; De Veer *et al.*, 1994; Hiskia *et al.*, 2001; Varghese *et al.*, 2007) (Fig. 3).

It also resisted degradation upon photolysis with plain UV light at 254 nm. However it has been reported to decompose upon photolysis with lambda > 200 nm. Combination of Ultrasound and photolysis with polyoxometalates produces only a cumulative effect (Dao *et al.* 2011; De Veer *et al.* 1994; Hiskia *et al.* 2001; Varghese *et al.* 2007).

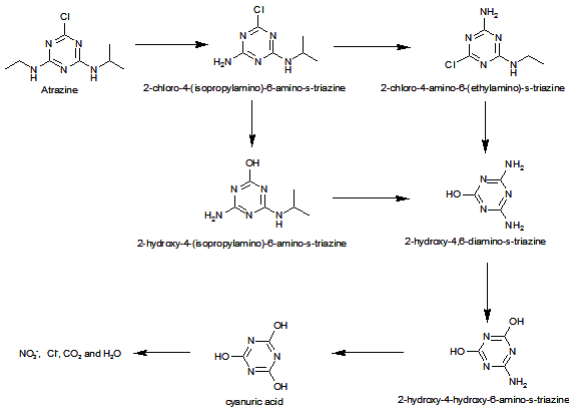


Fig. 3. Chemical (sonication, photolysis and peroxide) decomposition of atrazine.

Simultaneous application of microwave power and UV light leads to improved results in photochemical processes kinetic analysis showed that an indirect reaction of atrazine with an OH radical is dominant at low concentrations of H_2O_2 and a direct reaction of atrazine with H_2O_2 is dominant when the concentration of H_2O_2 is more than 200 mg/L (Chen *et al.*, 2011).

Biodegradation of atrazine

Atrazine is considered persistent due to its moderate water solubility decomposed metabolites halogen methylthioether and N-alkyl substituents on the s-triazine ring of this group of herbicides hinder the microbial metabolism (Wackett *et al.*, 2002) some reports have demonstrated the ability of some soil microorganisms to degrade atrazine partially or totally directing it to carbon dioxide and ammonia formation (Mandelbaun *et al.*, 1995; Rosseaux *et al.*, 2003; Singh *et al.*, 2004a). The biodegradation of atrazine in soil is space variable being slower in subsurface zones than in surface soil (Radosevich *et al.*, 1996). Repeated or overuse of atrazine can increase biodegradation which may be also enhanced as a result of limited N availability (Rhine *et al.*, 2003; Fang *et al.*, 2001). Silva *et al.*, (2004) demonstrated the occurrence of fast atrazine mineralization after an acclimatization period of approximately 28 days.

Among bacteria there are number of stains for the atrazine degradation such as Pseudomonas sp. (Katz *et al.*, 2001) Rodococcus rhodochrous (Jones *et al.*, 1998) Acinetobacter spp. Aerobacterium sp. Microbacterium sp. Bacillus sp. Micrococcus sp. Deinococcus sp. And Delftia acidovorans (Vargha *et al.*, 2005) as well as by species consortia including Agrobacterium tumefaciens Caulobacter crescentus Pseudomonas putida Sphingomonas yaniokuyae Nocardia sp. Rhizobium sp. Flavobacterium oryzihabitans and Variovorax paradoxus (Smith *et al.*, 2005). Pseudomonas sp. ADP isolated from soil contaminated with atrazine was shown to mineralize completely the triazinic ring (Mandelbaum *et al.*, 1995) (Fig. 4).

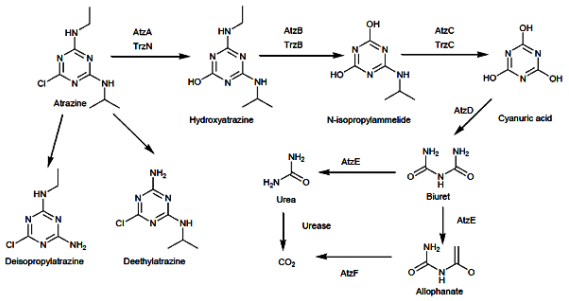


Fig. 4. Atrazine degradation pathways in bacteria.

The hydrolytic dechlorination initiation is the main atrazine degrading pathway bacteria generally catalysed by the enzyme atrazine chlorohydrolase (AtzA) encoded by the atzA gene followed by two hydrolytic deamination reactions catalysed by hydroxy-atrazine ethylamino-hydrolase (AtzB) and N-isopropylammelide isopropyl-amino-hydrolase (AtzC) encoded by the genes atzB (trzB) e atzC (trzC) respectively (De Souza *et al.*, 1996; De Souza *et al.*, 1998a; Sadowsky *et al.*, 1998) which convert atrazine sequentially to cyanuric acid that is then completely mineralized to CO_2 and NH_3 by other three hydrolases. In some bacterial strains the biodegradation of atrazine initiate through N-dealkylation of the lateral ethyl and isopropyl chains to deethylatrazine and deisopropylatrazine (De Souza *et al.*, 1998a; Sadowsky *et al.*, 1998).

Treatment/Removal and degradation atrazine from environmental medium

Practical and economical processes must be developed and applied in order to reduce the herbicide atrazine discharges into the environment. Physicochemical methods such as membrane filtration and adsorption using activated carbon have been applied to remove atrazine. However the main disadvantage of such methods is that they do not destroy the pollutants but transfer the pollutant from one phase to another (Dao *et al.*, 2011; De Veer *et al.*, 1994; Hiskia *et al.*, 2001; Varghese *et al.*, 2007). Nowadays advanced oxidation processes have been proposed as alternative methods for the elimination of atrazine among others biorecalcitrant compounds in wastewater (Dao *et al.*, 2011; De Veer *et al.*, 1994; Hiskia *et al.*, 2001; Varghese *et al.*, 2007). The principle of advanced oxidation processes (including O_3/H_2O_2 UV/O_3 UV/H_2O_2 H_2O_2/Fe^{2+} and $UV\text{-}TiO_2$) is to produce hydroxyl radical (by nucleophilic interactions) in water a very powerful oxidant capable of oxidizing a wide range of organic compounds with one or many double bonds (Dao *et al.*, 2011; De Veer *et al.*, 1994; Hiskia *et al.*, 2001; Varghese *et al.*, 2007). As reported hydroxyl radicals are produced from oxidizing agents such as ozone hydrogen peroxide often combined with UV radiation or semiconductor/metallic catalysts. An overview of the recent research studies using different processes for atrazine removal is summarized in Table 3.

Table 3. Most common methods of removal of atrazine from environment.

Method	Application & References
Covalent sequestration	Monochlorotriazines including atrazine and its major metabolites, deethylatrazine and deisopropylatrazine, are susceptible to nucleophilic aromatic substitution. Competitive reactions to rank the relative reactivity of nucleophiles with atrazine reveal that constrained secondary amines are the most reactive. When the nucleophile is attached to a solid support, atrazine can be sequestered from solution (Acosta *et al.* 2004).
Chemical degradation	Photolysis, hydrolysis, oxygenation (Ahalya *et al.* 2003).
Phytoremediation	Poplar trees seemed to be effective in rapid assimilation of ring leveled atrazine (90%) from sandy soil in less than 9 days (Burken *et al.* 1994).
De-oiled two phase Olive mill waste	Effects of de-oiled two-phase olive mill applied to soil for sorption (Antonio *et al.* 2010).
Ultrasonic destruction	The use of high power ultrasound to destroy pesticide contaminants like DDT, chlordane, atrazine, 2,4,5-T and endosulfan in sand slurries (Collings *et al.* 2010).
Photocatalytic degradation	Photocatalytic degradation of pyrene on soil surfaces using nanometer anatase TiO_2 under UV irradiation. The organic contaminants destroyed in a relatively short time when the contaminated soils containing atrazine, 2-chlorophenol, 2,7 dichlorodibenzodioxin mixed with TiO_2 and exposed to simulated solar radiation (Dong *et al.* 2010).
Dissipation	The dissipation of herbicide O-methyl-O-(2,4-dimethyl-6-nitrophenoxy)-Nisopropyl phosphoramidothioate in soil (Zhang *et al.* 2010).
Reverse osmosis	Impurity is separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by the dissolved solids (Ahalya *et al.* 2003).
Biodegradation	Degradation of atrazine by microorganisms like bacteria (*Pseudomonas* and *Agrobacterium tumefaciens*) and fungi (*Phanerochaete chrysosporium* and *Aspergillus fumigates*).

Membrane processes

The reverse osmosis nanofiltration and ultrafiltration are membrane processes (Ahalya *et al.*, 2003). The reverse osmosis constitutes one of the membranes processes that has been widely used in combination with nanofiltration (or ultrafiltration) to remove atrazine (Ahalya *et al.*, 2003). These studies revealed for example that reverse osmosis/ultrafiltration technique is considered as an effective method to remove atrazine (60-85%) from wastewater (Ahalya *et al.*, 2003).

Adsorption processes

In the adsorption process the pollutant is transferred from liquid phase to solid surface. Granular activated carbon is the most popular adsorbent used but its cost and the difficulties of regeneration represent the major drawbacks (Chen *et al.*, 2011; Ho *et al.*, 2011). Despite the fact that the adsorption is a well-known process this technology has not been widely used to remove atrazine (Brooks *et al.*, 2012; Castro *et al.*, 2009; Zadaka *et al.*, 2010). Adsorption using rubber carbon was applied by Gupta *et al.*, (2011) to remove atrazine (90% i.e. 90mg/g) from water. Furthermore Zhang *et al.*, (2013) analyzed the adsorption of atrazine on pig manure the observed adsorption was only 28% observed.

Different sorbents described earlier have been applied to remove atrazine. The physical–chemical properties (pH salinity temperature etc.) and the chemical composition of water or wastewater are main criteria in the selection of sorbents (Brooks *et al.*, 2012; Zheng *et al.*, 2010). Choosing the best sorbent for atrazine removal should take into account not only the efficiency but also the cost. From this point of view biosorbent generated from activated sludge could be an interesting alternative for atrazine removal. However the main disadvantage of such method is that they do not destroy the pollutant. The adsorption is a process to concentrate the contaminants (Zhang *et al.*, 2013).

Photochemical processes

In the recent years the use of UV light irradiations for the degradation of atrazine is one of the approaches that have been widely investigated. Atrazine and its metabolites are not very sensitive to the light irradiation. Photolysis processes using UV radiation are simple clean and less expensive. In direct photolysis UV radiations absorbed by H_2O molecule allow the generation of powerful oxidizing species such as the hydroxyl radicals (OH·) and the hydrogen peroxide (H_2O_2) (Chen *et al.*, 2011; De Veer *et al.*, 1994). To this edge researches focus on the combinations of UV radiations with oxidant species such as H_2O_2 and O_3 (UV/H_2O_2 and UV/O_3). Ozonation technique was applied to remove atrazine from water and livestock wastewater respectively (Wert *et al.* 2009). The ozone is a strong oxidant capable to act direct or indirectly with pollutants. Otherwise the presence of hydroxide ions could initiate the decomposition of ozone in water to form hydroxyl radicals (da Silva *et al.*, 2009). Another pathway to enhance the ozonation performance is to combine O_3 with UV irradiation; the photolysis of ozone produces hydrogen peroxide which initiates the further decomposition of residual ozone into hydroxyl radical. According to the results reported above the efficiency of the ozonation process on the removal of atrazine showed a difference. The operating conditions imposed during the treatment (pH atrazine dose dose of ozone mixing inside the reactor etc.) and the mass transfer limitations are a relevant factor to be considered in the oxidation process with ozone (Barreiro *et al.*, 2007; Dao and De Laat 2011). It is worth noting that the presence of organic matter suspended solids carbonate/bicarbonate and chlorine ions could also affect the performance of ozone process (Anipsitakis and Dionysiou 2003; De Laat *et al.*, 2004). As far as the high cost of the equipment and maintenance as well as the energy required to supply the process constitutes one of the disadvantages of the ozonation technique. Photo-Fenton process ($UV/Fe^{2+}/H_2O_2$) is another attractive oxidation system because it uses low cost reagents iron is abundant and a non-toxic element and

hydrogen peroxide is easy to handle and environmentally safety. The use of UV radiation increases the efficiency of the oxidation process due to the highly generation of hydroxyl radicals by the photolysis of ferric complexes and the regeneration of ferrous ions (Du *et al.*, 2011 2012). Atrazine was removed from different water sources (Spiked wastewater treatment plants effluent surface and deionized water) under solar irradiation. Under these conditions the average mineralization rate recorded in wastewater treatment plants surface water and deionized water were 71 87 and 90 % respectively. These results suggest that the radiation may have been attenuated by the organic matter in the wastewater treatment plants matrices. The photolysis of aqueous $Fe(OH)^{2+}$ has been hindered and consequently the generation of OH radicals as limited. The high chemical consumption which sometimes produces a secondary pollution and a huge volume of sludge and the relatively higher treatment cost constitutes another major drawback for large scale application of these processes (Dao and De Laat 2011; Hiskia *et al.*, 2001).

Electrochemical process

Nowadays electrochemical technologies applied for the treatment of atrazine have received considerable attention in the environmental field. Electrochemical method combining chemistry and electronic science (electron transfer) has widely proved to be a clean flexible and powerful technique for water and wastewater treatment. Electrochemical treatment is characterized by simple equipment easy operation safety selectivity environmental compatibily and brief retention time (Hromadová *et al.*, 2006; Pospisil *et al.*, 1996). Compared to chemical oxidation no addition of chemicals is necessary in the process of electrochemical degradation. In electrochemical oxidation processes pollutant can be removed electrochemically by a direct anodic oxidation where pollutant are firstly adsorbed on the anode surface and then destroyed through the anodic electron exchange (Macyk *et al.*, 2003). On the other hand pollutants could be also degraded indirectly in the liquid bulk through reactive oxidant species (OH O_3 H_2O_2 chlorinated species etc.) which act as intermediates for electrons transference between the electrode and the refractory organic compounds (Hromadová *et al.*, 2006; Pospisil *et al.*, 1996). This technology has been not widely applied to remove atrazine antibiotics under different experimental conditions there was only few studies have been reviewed.

There is several authors studied number of methods which have been developed recently for the removal and degradation atrazine from environmental medium. Developments of sensitive and economic analytical methods are very crucial for screening the quantitative presence of atrazine and preventing toxicological risks. In general gas chromatography (GC) and high performance liquid chromatography (HPLC) are the techniques popularly used for the determination of atrazine and simazine (Usenko *et al.*, 2005; Stalikas Knopp & Niessner 2002; Koal *et al.*, 2003; Baranowska Barchanska & Pacak 2006). Gas chromatography–mass spectrometry (GC-MS) amperometric immunosensr adsorptive stripping voltammetric determination were developed for the analysis of atrazine and simazine (Maleki *et al.*, 2007; Grennan *et al.*, 2003; Turiel *et al.*, 1998; Nevado *et al.*, 2007) UV-visible and NMR.

Analytical Methods to Determine Atrazine in Biological Media

Preparation and Pretreatment

There are number of methods have been described for the analysis of different class of pesticides in different biological media (Kumar and Upadhyay, 2013; Kumar, 2013, Kumar *et al.*, 2013a, b & c, and 2014; Prasad *et al.*, 2013). Though atrazine is belongs to polar pesticide and highly soluble in polar solvents like acetone methanol and water so there is pretreatment step become more important (Tariq *et al.*, 2010; Zayed *et al.*, 2008). Matrix preparation and quality control of atrazine has been reported by collecting the soil samples i.e. collected in the field are commonly air-dried grounded and sieved through a

mesh with a grain size of 2 mm (Vicente and Yolanda 2004). One basic requirement is to assess how much analyte has been removed from soil by the selected extraction technique (Vicente and Yolanda 2004). Extraction techniques are involved as Liquid–solid extraction (prominently Soxhlet based technique) basically performed by using water/acetone as extractant for atrazine because of polarity factor (Vicente and Yolanda 2004). Next step is clean up of atrazine this step performed to avoid interference with other organic motifs by using large number of sorbents for isolation of organic compounds from the extracted solutions including alumina Florisil ion-exchange resins silica gel many silica-based sorbents (e.g. octadecyl- octyl- phenyl- and diol-bonded silica) and graphitized black carbon (Vicente and Yolanda 2004) (Fig. 5).

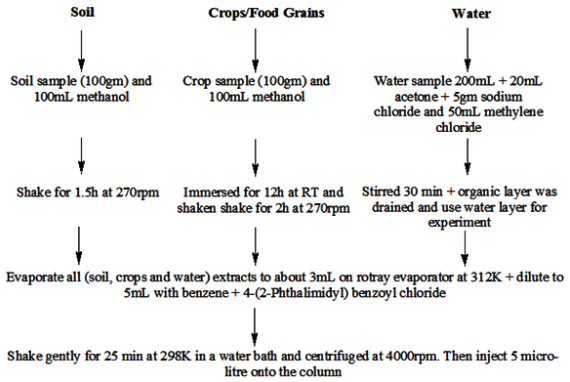


Fig. 5. Most probable extraction, cleanup and sample loading steps of atrazine.

Determination of Atrazine in Milk or in Dietary Product

Atrazine accumulation in milk-producing animals (from contaminated feed and water) has been monitored through the simple tube enzyme-linked immunosorbent assay (ELISA) procedure. Comparatively samples were extracted with hexane-acetone for HPLC analysis. A good correlation between 1% and 2% was found between the two methods in the analysis of real samples. However it has been observed that the ELISA procedure was more sensitive that the HPLC method since atrazine was detected in some samples by the ELISA but was not confirmed by the HPLC method (Barchanska *et al.*, 2012).

Determination of Atrazine in Soil Crop and Water Samples

Coupled off-line extraction a pre-column derivatization liquid chromatographic fluorescence detection procedure has been developed for the determination of atrazine in soil crop and water samples. 4-(2-Phthalimidyl) benzoyl chloride (PIB-Cl) was used as a pre-column derivatization reagent for high performance liquid chromatography. Detection limit of 1.2 ng/g for atrazine was obtained with recoveries of 84–95% for environmental samples (Gong *et al.*, 1998). Two-dimensional gas chromatography and liquid chromatography coupled with time of flightmass spectrometry have been used to examine metabolite profiles of *Hyalella azteca* chronically exposed to 30 µg/L atrazine and DEA. It has been observed that the majority of identified metabolites were by-products of β-oxidation of fatty acids suggesting possible disruption in energy metabolism. Eicosanoids increased in exposed females suggesting possible perturbations in neuropeptide hormonal systems. Overall research demonstrates the feasibility of utilizing metabolomic profiling of invertebrate species exposed to environmental contaminants as a way to determine mechanisms of toxicity (Ralston-Hooper *et al.*, 2012). Reverse phase HPLC method has been develop for multicomponent pesticides trace residues in few gram soil samples with good LOD 10-50 ng/50g of soil good recovery range ranged from 85 to 98% and with acceptable reproducibility (Hutta *et al.*, 2009; Di Corcia *et al.*, 1987). In situ optical sensor (consists of a polymer-coated attenuated total reflection (ATR) element or a silver halide optical fiber) for monitoring atrazine in water has been developed Absorbance data were recorded for atrazine at 1577 cm^{-1} and observable limit of detection found in the region of 2 ppm (Regana *et al.*, 1996).

Determination of Atrazine in Human and Animal Tissues

A high performance liquid chromatography method has been used to study the plasma kinetics of atrazine in a human fatality after ingestion of a herbicide mix containing atrazine aminotriazole ethylene glycol and formaldehyde. A hemodialysis was performed in an effort to eliminate these toxic substances. The mean atrazine clearance over 4 h was 250 mL/min and the dialysance of atrazine was calculated as 76%. On autopsy the kidney showed the highest concentration of atrazine (97.62 micrograms/g-1 wet tissue) with lesser concentrations in the lung small intestine and liver and the lowest concentration in the heart (Pommery *et al.*, 1993). A sensitive LC-MS method for the analysis of atrazine and its metabolites in mouse urine and plasma has been developed; the main aim of the study was to detect and measure simultaneously atrazine and its major metabolites in the mammalian. Didealkyl atrazine was the most abundant metabolite detected in the urine and plasma samples (approximately 1000 μM in 24-h urine and approximately 100 μM in plasma (Matthew *et al.* 2009; Ross *et al.*, 2006).

Microwave-assisted extraction of sheep liver using methanol as extractant and analysis of extracts by high performance liquid chromatography and ultraviolet detection has been developed The recoveries of the method at two different spiked levels were assessed by analyzing spiked liver samples and were found to be in the range from 90 to 102% with good precision (<11%) (Cheng *et al.*, 2007). A two-dimensional high performance liquid chromatography separation and tandem mass spectrometry detection method for the detection of atrazine and its metabolic and hydrolysis products in urine has been developed. The 2D-HPLC system incorporated strong cation exchange and reversed phase separation modes. This versatile approach can be used for the quantitative determination of all 12 compounds (metabolites of atrazine) in experimental animals for toxicological studies. The method requires only 10 μL of urine and the limits of detection (LODs) range from 10 to 50μg/L. The method can also be applied to assess atrazine exposure in occupational settings by measurement of 6-Cl and 6-Mer analogs which requires only 100μL of urine with LODs of 1-5μg/L. Finally in combination with automated off-line solid phase extraction before 2D-HPLC the method can also be applied in non-occupational environmental exposure studies for the determination of 6-Cl and 6-Mer metabolites using 500 μL of urine and LODs of 0.1-0.5 μg/L (Kuklenyik *et al.*, 2012).

Future Trends

This paper underlines the worldwide usage of atrazine fate and toxicity in the aquatic and terrestrial environment. On one hand the cheapest cost makes it more suitable for agriculture sector; and on the other hand it also makes it potentially significant as environmental contaminants due to its higher stability and persistence in biological media. Studies found that the average degradation half live of atrazine varied from 60-180 days in different biological media and depends upon the cofactors of relevant conditions (pH chelation and photo-degradation). Workers and researchers analyzed whether the degradation products were potent to environmentally relevant sludge and soil bacteria.

Besides reported concentrations of atrazine in different environmental compartments are still detected in lower trace levels and ultimately depend on spatial and climatic variations. Given this fact techniques developed for the detection cannot be extrapolated. In this context analytical method for the detection should have higher sensitivity selectivity and specificity. It is critical to find multi-residual method to detect atrazine and its by-products. HPLC and liquid chromatography coupled to mass spectrometry which provides new opportunities for analysis of atrazine in water is highly recommended. Liquid chromatography coupled to mass spectrometry has been developed and effectively implemented over conventional detection method such as UV and fluorescence.

It should be also mentioned that several questions remain unanswered for the conventional treatment processes (biological or physico-chemical treatments) generally applied to remove atrazine through wastewater treatment plants. The occurrence of atrazine in the aquatic and terrestrial environmental is mainly due to the unsuccessful of conventional treatment processes (coagulation sedimentation UV irradiation and biological) applied in wastewater treatment plants in the removal of these compounds. To overcome this drawback novel approaches that use and integrate the chemical data including advanced oxidation processes have been developed and applied to remove atrazine from water wastewater soil and sludge. Until date applications of advanced oxidation processes for atrazine removal were carried out at the laboratory scale. Future research should be focused on the development of advanced oxidation processes for large scale applications. Advanced oxidation processes are one of the most powerful processes for the removal of pesticides from the environment. In addition these processes can be effectively used to remove by-products toxicity and enhance the mineralization rate of atrazine. The microorganisms used in biological process are sensible to the toxic pollutants. Thus advanced oxidation processes could be applied as pre-treatment step in which the pollutant are oxidized to by-products that are easily biodegradable and less toxic. This combined process avoids the death of microorganisms that are present in biological treatment. Likewise coupling a biodegradation process with physicochemical process would improve atrazine removal and reduce the operating cost.

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